arginines and an insertion of the truncated Factor XI intron 1 (SEQ. ID NO: 9) in one or more splice sites of the Factor VIII cDNA;

b) introducing the modified Factor VIII cDNA into a cell; and

c) expressing the modified cDNA in said cell to produce Factor VIII protein.

11. **(Amended)** A process for producing a Factor VIII protein comprising:

a) obtaining a wildtype cDNA of the Factor VIII protein;

b) introducing splice sites into the wildtype Factor VIII cDNA;

c) preparing a modified Factor VIII cDNA by inserting one or more introns into the wildtype Factor VIII cDNA splice sites;

d) introducing the modified Factor VIII cDNA into a cell; and

e) expressing the polypeptide encoded by the modified Factor VIII cDNA in the cell to produce the protein,

wherein the yield of the protein produced with modified Factor VIII cDNA is greater than the yield produced with wildtype Factor VIII cDNA.

IN THE DRAWINGS:

Subject to the approval of the Examiner, please append Figures 1-7 to this application.

REMARKS

Upon entry of the Amendment and Response, claims 7-11 are pending in this application. Claims 7 and 8 have been amended to correct informalities objected to by the Examiner. Claim 11 has been amended to more precisely

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point out the subject matter being claimed. Support for these amendments can be found on page 2, lines 4-8, and on page 12, lines 3-19, disclosing the protein of interest to be Factor VIII protein. Claim 11 and the specification have been amended to correct typographical errors.

Figures 1-7, which were submitted in parent application 09/526,935, which was incorporated by reference herein are now submitted in this application. Accordingly, no new matter has been added.

Priority

The Office has stated that "the parent application upon which priority is claimed fails to provide adequate support for Claim 11 of this application." (*See* Paper No. 8, page 3.) Applicants have amended Claim 11 such that the claim is now supported by the specification of parent application 09/526,935, on which we rely for priority.

The Office specifically requested page citations supporting the concept of introducing splice sites into a wildtype cDNA. Page 4, lines 1-20, of the parent specification describe the introduction of a splice site into a wildtype Factor VIII cDNA. This disclosure provides support for the amended claim because applicants now specifically claim introducing splice sites, such as the Sal I site disclosed in the parent specification, into Factor VIII wildtype cDNA.

The Office also requested page cites supporting the concept of preparing a modified cDNA by inserting one or more introns into the splice sites. Page 5, lines 10-25, of the parent specification disclose the insertion of the FIX truncated intron 1 into the splice site. This disclosure provides support for the amended

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claim because applicants now specifically claim inserting introns, such as the FIX truncated intron 1, into the splice sites opened in Factor VIII cDNA.

Accordingly, each limitation of claim 11 is supported by the parent specification. Thus, claim 11 is entitled to a priority date of March 17, 1999.

Rejection Under 35 U.S.C. § 112, First Paragraph

The Office has rejected claim 11 under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. (*See* Office Action, page 4.) Applicants have amended Claim 11 to claim a process for producing a Factor VIII protein, rather than "any wild-type cDNA encoding a protein." The specification discloses three examples of the claimed process for producing a Factor VIII protein from a modified Factor VIII cDNA, each of which demonstrates a higher protein yield than does wild-type Factor VIII cDNA. (*See* Specification, Page 5, lines 10-25; Figure 3.) This disclosure in the specification conveys that the inventors were in possession of the claimed invention. Accordingly, the rejection should be withdrawn.

The Office has also rejected claim 11 under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification sufficiently to enable one skilled in the art to make or use the invention. (*See* Office Action, page 6.) Applicants have amended claim 11 to claim a process for producing Factor VIII protein, rather than "any wild-type

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cDNA encoding a protein." By amending the claim, applicants have claimed subject matter fully enabled by the specification.

The specification describes the process for producing a Factor VIII protein using three different cDNAs. (*See* Specification, page 5, lines 10-25.) These representative examples provide a high level of guidance such that there will be no undue experimentation.

Further, claim 11 has been amended to claim a process for producing a Factor VIII protein. The Office has indicated that even within the examples recited in the specification, there is a large degree of variability in Factor VIII protein production. (*See* Office Action, page 9.) Nonetheless, each modified Factor VIII cDNA construct produces more protein than the wildtype, meeting the limits of the claim. (*See* Figure 3). Accordingly, the rejection should be withdrawn.

Rejection Under 35 U.S.C. §112, Second Paragraph

The Office has rejected claim 11 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which applicant regards as the invention. Applicants have amended Claim 11 to claim a process for producing Factor VIII protein, rather than "any wild-type cDNA encoding a protein." By identifying the protein being produced and specifying that the splice sites of b) are the places where the intron or introns of c) are inserted, applicants have pointed out and more distinctly claimed the subject matter of the invention. Accordingly, the rejection should be withdrawn.

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Rejection for Obvious-type Double Patenting

The Office rejected claims 7-10 under the doctrine of obviousness-type double patenting, stating that the claims are not patentably distinct from claims 1-6 of U.S. Patent No. 6,271,025. Applicants have filed a terminal disclaimer in compliance with 37 CFR 1.321 (c). Accordingly, the rejection should be withdrawn.

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON,
FARABOW,
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Dated: December 23, 2002

By: _____
Sanya Sukduang
Reg. No. 46,390

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APPENDIX

In the Abstract

Amend the abstract as follows:

A modified Factor VIII cDNA is described in which the B-domain of the wild-type Factor VIII cDNA has been deleted and a truncated Factor IX intron has been inserted in one or more **[locatons]** **locations** of the Factor VIII cDNA. Such modified Factor VIII cDNA may be used for a higher yield production of Factor VIII in vitro as well as in a transfer vector for gene therapy.

In the Specification

Please amend the specification as follows:

Please amend page 2, lines 4-8, as follows:

According to this invention, a modified FVIII cDNA is made available in **[wich]** **which** the B-domain of the wild-type FVIII cDNA has been deleted and a truncated FIX intron 1 has been inserted in one or more locations of the FVIII cDNA. In addition the B-domain of the wild-type FVIII cDNA has been replaced by four arginines.

Please insert the following on page 2, following line 8:

BRIEF DESCRIPTION OF THE FIGURES

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FIG. 1. Schematic view of the Factor IX intron I inserted in the Factor VIII intron 1 location.

FIG. 2. Cloning Strategy of Factor IX intron 1 in Factor VIII introns 12 and 13 locations.

FIG. 3. Kinetic Factor VIII production from different transfected cell lines.

FIG. 4. Procoagulant activities from different transfected cell supernatants.

FIG. 5. Factor VIII antigen production in a Hep G2 cell line.

FIG. 6. Quantitative analysis of Factor VIII mRNA in transfected Chinese Hamster Ovary cells.

FIG. 7. Intracellular amount of Factor VIII in different cell lines.

Please amend page 3, lines 9 through 11, as follows:

WT sequence: TAA GTC ATG CAA ATA **(SEQ. ID NO. 10)**

Kozak modified: ACA **CCC** ATG **G**AA ATA **(SEQ. ID No. 11)**

The modified **[amino] nucleic** acids are represented in bold.

Please amend page 4, lines 1 through 13, as follows:

Four arginines replace according to the invention the B-domain of the FVIII protein. They are introduced by the oligonucleotides used for the cloning of the two fragments surrounding the B-domain (see Fragments 2 and 3 of Table 1), namely the oligonucleotides 4R AS (SEQ. ID No. 4) and 4R S (SEQ. ID No. 5). The Sal I site was generated by the coding sequence of the arginines as follows:

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SAL I SITE

4R S :5'-A AGA CGT CGA CGA GAA ATA ACT CGT ACT ACT CTT **(SEQ. ID No. 5)**

4R AS TTG TTA CGG TAA CTT GGT TCT GCA GCT GCT CTT **(SEQ. ID No. 4)**

CORRESPONDING PEPTIDIC SEQUENCE:

Pro Arg Arg Arg Arg Glu Ile Thr Arg Thr Thr Leu **(SEQ. ID No. 12)**

In the wild-type FVIII the peptidic sequence is:

Pro-Arg-Domain B-Arg-Glu **(SEQ ID No. 13)**

Please amend page 5, lines 11 through 13, as follows:

According to the invention, the FVIII cDNA was further modified by the insertion of a Factor IX truncated intron 1 **[(FIX TI1 SEQ. ID No. 9)]** **(FIX TI1) (SEQ. ID No. 9)**. The FIXTI 1 was inserted in different locations of the FVIII cDNA as follows:

Please amend page 6, lines 1 through 5, as follows:

The **[FIXT1I-Sequence (=SEQ-ID No. 9)]** **FIX TI1 sequence (SEQ. ID No. 9)** used according to the invention in different locations of the FVIII cDNA starts after the coding sequence by the splice donor sequence and ends by the splice acceptor sequence of the truncated intron 1. The upper case letters start after and stop before the Nsi I and Mlu I restriction sites, respectively. **[For details, see Annex 2.]**

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Please amend page 6, lines 10 through 22, as follows:

A similar strategy was used for inserting the three **[FIXTI1]** **FIX TI1** in different locations. In each case three PCR fragments (A,B,C) were generated with the Expand **[System]** **system** using Factor VIII cDNA as a template for the segments A and C, and Factor IX intron 1 for the B fragment. The A fragment extremities comprise Factor VIII sequence on the 5' end, and on the 3' end a fusion between the FVIII 3' splicing sequence and the Factor IX first intron 5' splicing sequence. A Nsi I restriction site was added between these two sequences. The B fragment possesses at the 5' extremity a complementary sequence to the previous fragment, the truncated Factor IX intron 1, and at the 3' end and inserted **[MluI]** **Mlu I** restriction site. The C fragment was made of the complementary sequence of the 3' extremity from fragment B followed by the Factor IX first intron 3' splicing sequence and by the Factor VIII cDNA downstream coding sequence (see Figure 1).

Please amend page 11, lines 2 through 21, as follows:

A sheep anti-FVIII antibody from Cedarlane (Hornby, Canada) was purchased and positively tested in a control immunoblot using recombinant FVIII. This antibody was used in an immunoblot on cell supernatant but no signal was obtained due to the low amount of secreted antigen. An immunoprecipitation was done on cell supernatant but here again no signal was obtained indicating the inability of this antibody to immunoprecipitate FVIII. An immunoblot was

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done **[o]** **on** Triton-X100 soluble cell lysates. 90mm dishes **[was]** **were** lysed with 300 **[l]** **ml** ice-cold lysis buffer (Hepes 20 mM pH7.5, KCl 100mM, $MgCl_2$ 2mM, Triton-X 100 0.5 %). Cells were **[scrapped]** **scraped** and centrifuged at 4 C, 10 min at 14000g. Protein concentration was measured with the Dc-Protein Assay kit (BioRad, Hurcules, USA). 175 g of each cell **[lysates]** **lysate** were loaded on a 7.5% acrylamide gel and treated following the Laemmli protocol. After **[semy-dry]** **semi-dry** transfer (35 min at 400mA), the nitrocellulose membrane was incubated overnight in TBS-T (20 mM Tris pH 7.5, NaCl 0.15 M, Tween-20 0.5%). The membrane was then incubated 1 h with the anti-FVIII antibody (5 g/ml) in TBS-T. After three washes of 10 min each in TBS-T, the membrane was incubated for 30 min with a rabbit anti-sheep peroxidase coupled antibody (dilution 10^{-4} in TBS-T). Extensive washes were conducted before revelation with the ECL system (Amersham).

Please amend page 12, lines 3 through 19, as follows:

[Subject] **Subjects** of the invention are, therefore, FVIII-B-domain deleted constructs containing a **[slightyl]** **slightly** modified Factor IX truncated intron I in different locations of the cDNA. Among these constructs a cDNA bearing the truncated intron I in both the FVIII Intron 1 and Intron 12 locations led to a 100 times higher intracellular production than all other constructs and a 9 times higher secretion of the protein. This improved production and secretion was observed in two different cell lines: CHO and HepG2 cells. The FVIII produced was fully active on a one stage clotting assay and appears homogenous on

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immunoblot. The mRNA amount of all the constructs tested differs no more than three times indicating that the benefit observed in the production is coming both from a transcriptional and a translational effect.

In the Claims

Please amend claims 7, 8 and 11 as follows:

7. A process for the production of Factor VIII protein comprising:

a) preparing a modified Factor VIII cDNA, **[comprising a] wherein said Factor VIII cDNA is modified by** deletion of the B-domain and an insertion of the truncated Factor XI intron 1 (SEQ. ID NO: 9) in one or more splice sites of the Factor VIII cDNA;

b) introducing the modified Factor VIII cDNA into a cell; and

c) expressing the modified cDNA in said cell to produce Factor VIII protein.

8. A process for the production of Factor VIII protein comprising:

a) preparing a modified Factor VIII cDNA, **[comprising a] wherein said Factor VIII cDNA is modified by** replacement of the B-domain with nucleotides encoding four arginines and an insertion of the truncated Factor XI intron 1 (SEQ.ID NO: 9) in one or more splice sites of the Factor VIII cDNA;

b) introducing the modified Factor VIII cDNA into a cell; and

c) expressing the modified cDNA in said cell to produce Factor VIII protein.

11. A process for producing a **Factor VIII** protein comprising:

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a) obtaining a wildtype cDNA of the **Factor VIII** protein;

b) introducing splice sites into the wildtype **Factor VIII** cDNA;

c) preparing a modified **Factor VIII** cDNA by inserting one or more introns **[from one or more additional cDNAs]** into the wildtype **Factor VIII** cDNA **splice sites**;

d) introducing the modified **Factor VIII** cDNA into a cell; and

e) expressing the polypeptide encoded by the modified **Factor VIII** cDNA in the cell to produce the protein,

wherein the yield of the protein produced with modified **Factor VIII** cDNA is greater than the yield produced with wildtype **Factor VIII** cDNA.

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